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Upregulation of NOS by simulated microgravity, potential cause of orthostatic intolerance

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Vaziri, N. D., Y. Ding, D. S. Sangha, and R. E. Purdy. Upregulation of NOS by simulated microgravity, potential cause of orthostatic intolerance. *J Appl Physiol* 89: 338–344, 2000.—Prolonged exposure to microgravity during spaceflight or extended bed rest results in cardiovascular deconditioning, marked by orthostatic intolerance and hyporesponsiveness to vasopressors. Earlier studies primarily explored fluid and electrolyte balance and baroreceptor and vasopressor systems in search of a possible mechanism. Given the potent vasodilatory and natriuretic actions of nitric oxide (NO), we hypothesized that cardiovascular adaptation to microgravity may involve upregulation of the NO system. Male Wistar rats were randomly assigned to a control group or a group subjected to simulated microgravity by hindlimb unloading (HU) for 20 days. Tissues were harvested after death for determination of total nitrate and nitrite (NOx) as well as endothelial (e), inducible (i), and neuronal (n) NO synthase (NOS) proteins by Western blot. Separate subgroups were used to test blood pressure response to norepinephrine and the iNOS inhibitor aminoguanidine. Compared with controls, the HU group showed a significant increase in tissue NOx content and an upregulation of iNOS protein abundance in thoracic aorta, heart, and kidney and of nNOS protein expression in the brain and kidney but no discernible change in eNOS expression. This was associated with marked attenuation of hypertensive response to norepinephrine and a significant increase in hypertensive response to aminoguanidine, suggesting enhanced iNOS-derived NO generation in the HU group. Upregulation of these NOS isotypes can contribute to cardiovascular adaptation to microgravity by promoting vasodilatory tone and natriuresis and depressing central sympathetic outflow. If true in humans, short-term administration of an iNOS inhibitor may ameliorate orthostatic intolerance in returning astronauts and patients after extended bed rest.

nitric oxide synthase; syncope; orthostatic hypotension; spaceflight; weightlessness; astronauts

PROLONGED EXPOSURE TO MICROGRAVITY during spaceflight or extended bed rest on Earth results in cardiovascular deconditioning, which is marked by orthostatic intolerance and impaired exercise capacity on reexposure to gravity (6, 52). Gravitational forces on Earth promote a normal shift of the extracellular fluids to the lower parts of the body. The effect of gravity on extracellular fluid distribution is evidenced by the presence of a pronounced gradient in mean arterial blood pressure between the head (~70 mmHg) and feet (~200 mmHg) during upright posture in normal humans on Earth (12). Exposure to microgravity leads to an immediate redistribution of extracellular fluid to the upper half of the body, as evidenced by the rise in mean arterial blood pressure in the head, from ~70 mmHg on Earth to ~100 mmHg in space (12). This results in an early rise and a later fall in central venous pressure and development of hypovolemia, resting tachycardia, and diminished stroke volume in chronic phase (52). The upward shift in extracellular fluid distribution appears to be responsible for the cardiovascular adaptation to microgravity. This viewpoint is supported by the observation that a cardiovascular deconditioning similar to that seen in returning astronauts occurs in humans kept in a supine position with a 6% head-down tilt on Earth (52).

Under normal conditions, assumption of the upright position is accompanied by a significant vasoconstriction in the lower extremities. This helps to maintain blood pressure and flow in the upper part of the body, including the brain, by mitigating the gravitational shift of blood to the lower parts of the body. However, the physiological response is impaired in microgravity-adapted individuals, such as the returning astronauts who experience marked orthostatic hypotension and an increased propensity to syncope (52). This is, in part, due to hypovolemia and impaired vasoconstrictive response in such individuals (5).

Hindlimb unloading (HU) in rodents has been used as a model to simulate cardiovascular deconditioning in humans. This model exhibits many of the known cardiovascular consequences of microgravity in humans, including extracellular fluid redistribution, altered central venous pressure, hypovolemia, and reduced exercise capacity (8). Likewise, the HU animals exhibit a depressed vasoconstrictive response to norepinephrine (31) and other vasoconstrictors (8).

Nitric oxide (NO) is an endogenous modulator, which is produced by various cell types in different tissues

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and which regulates numerous biological processes. For instance, NO is the most potent endogenous vasodilator and as such plays an important role in the regulation of renal and systemic vascular resistance, tissue perfusion, and renal hemodynamics. In addition, NO inhibits renal tubular sodium reabsorption (17–19, 21, 30, 32, 33, 36, 37, 44, 45) and as such contributes to sodium and extracellular fluid volume homeostasis. Through these mechanisms, NO plays an important role in the regulation of blood pressure.

In an earlier study, we found functional evidence for an HU-induced increase in vascular NO activity (34). This was based on the observation that endothelium-mediated vasodilatory activity was increased in carotid arteries and the vasodilatory response to L-arginine was increased in the femoral arteries of the HU rats. Moreover, the vasodilatory response to L-arginine could be blocked by the inducible nitric oxide synthase (iNOS)-selective inhibitor, aminoguanidine, pointing to a possible rise in vascular iNOS activity. These observations led to the hypothesis of the present study that extended exposure to microgravity can lead to a general upregulation of the NO system. This hypothesis was addressed using the HU rat to simulate microgravity.

METHODS

Animal Model

The protocol employed in this study was approved by the Institutional Animal Care and Use Committee of the University of California, Irvine. Male Wistar rats weighing 250–300 g (Simonsen Laboratories, Gilroy, CA) were housed in a climate-controlled room (22°C) with a 12:12-h light-dark cycle. Water and rat chow were provided ad libitum. Animals were randomly assigned to control or HU groups. HU was achieved by the use of a tail harness that partially elevated the hindlimbs above the floor of the cage (31, 39). Briefly, the tail was cleaned, and a coat of benzoin tincture was applied and allowed to air dry until tacky. Adhesive strips (Fas-Trac of California, Van Nuys, CA) the width of the tail were then looped through a swivel harness and pressed along the sides of the tail to form a tubular casing around the tail. Thereafter, the tail was wrapped with Electoplast bandage (Beiersdorf, Norwalk, CT) followed by a thin layer of plaster cast material (Sammons Preston, Bolingbrook, IL). The rat was suspended by the swivel harness from a hook at the top center of the suspension cage, allowing a free 360° rotation. The height of the hook was adjusted such that the front limbs were in contact with the floor, and the hindlimbs were elevated ~0.5 cm above the floor when fully extended, tilting the body of the rat to an angle of 35° with the floor of the cage. The animals were exposed to HU for 20 days. At the conclusion of the 20-day observation period, subgroups of HU and control animals ($n = 6$ in each group) were used for determinations of blood pressure response to norepinephrine and aminoguanidine as described below. A second subgroup of six HU animals and six control rats were killed, and tissues were harvested for determination of NOS isotypes.

The rats were killed by exposure to 100% CO_2 for 90 s to induce deep anesthesia (10). The chest and abdomen were opened, and the heart, thoracic aorta, kidney, and brain were removed, cleaned in PBS, snap frozen in liquid nitrogen, and stored at −70°C until processed.

Response to Norepinephrine and Aminoguanidine

Under general anesthesia with Inactin (100 mg/kg ip), the left jugular vein and carotid artery were cannulated with polyethylene tubes (PE-50). The animal was placed on a heating pad, and arterial blood pressure was monitored directly via the arterial catheter that was connected to a Gould P-50 pressure transducer and recorded on a Dynograph R511A recorder (Sensor Medics, Anaheim, CA). Once stable, blood pressure was continuously recorded for 5 min to determine the baseline value. Subsequently, pressor responses to bolus injections of norepinephrine (0.15 μg/kg; Sigma Chemical, St. Louis, MO) and the iNOS inhibitor aminoguanidine (30 mg/kg; Sigma Chemical) were determined. Response to each drug was calculated as peak change in blood pressure from the baseline value. Mean arterial pressure was calculated as the sum of diastolic blood pressure and one-third of the pulse pressure. Each drug was injected at least twice, and the average of the values obtained was used. A 30-min recovery period was allowed after each bolus injection of norepinephrine. However, the aminoguanidine injections were separated by a 60-min interval.

NOS Protein Measurements

Homogenates (25% wt/vol) of kidney, heart (left ventricle), thoracic aorta, and brain were prepared in 10 mM HEPES buffer, pH 7.4, containing 320 mM sucrose, 1 mM EDTA, 1 mM dithiothreitol (DTT), 10 μg/ml leupeptin, and 2 μg/ml aprotinin at 0–4°C with the aid of a tissue grinder fitted with a motor-driven ground glass pestle. Homogenates were centrifuged at 12,000 *g* for 5 min at 4°C to remove nuclear fragments and tissue debris without precipitating plasma membrane fragments. The supernatant was used for determination of NOS isotype proteins. Total protein concentration was determined by using a kit from Bio-Rad Laboratories (Hercules, CA).

Endothelial NOS (eNOS), neuronal NOS (nNOS), and iNOS proteins were measured by Western blot analyses using anti-eNOS, anti-nNOS, and anti-iNOS monoclonal antibodies (Transduction Laboratories, Lexington, KY) in a manner the same as that previously described by Vaziri et al. (48, 49). Briefly, aorta, kidney, heart, and brain tissue preparations (50 μg of protein for aorta and brain and 100 μg for kidney and heart) were size-fractionated on 4–12% Tris-glycine gel (Novex, San Diego, CA) at 120 V for 3 h. In preliminary experiments, we had found that the given protein concentrations were within the linear range of detection for our Western blot technique. After electrophoresis, proteins were transferred onto Hybond-enhanced chemiluminescence (ECL) membrane (Amersham Life Science, Arlington Heights, IL) at 400 mA for 120 min using the Novex transfer system. The membrane was prehybridized in 10 ml of *buffer A* (10 mM Tris-hydrochloride, pH 7.5, 100 mM NaCl, 0.1% Tween 20, and 10% nonfat milk powder) for 1 h and then hybridized for an additional 1-h period in the same buffer containing 10 μl of the given anti-NOS monoclonal antibody (1:1,000). The membrane was then washed for 30 min in a shaking bath, changing the wash buffer (*buffer A* without nonfat milk) every 5 min before the 1-h incubation in *buffer A* plus goat anti-mouse IgG-horseradish peroxidase at the final titer of 1:1,000. Experiments were carried out at room temperature. The washes were repeated before the membrane was developed with a light-emitting nonradioactive method using ECL reagent (Amersham Life Science). The membrane was then subjected to autoluminography for 1–5 min. The autoluminographs were scanned with a laser densitometer (model PD1211, Molecular Dynamics, Sunnyvale,

CA) to determine the relative optical densities of the bands. In all instances, the membranes were stained with Ponceau stain, which verified the uniformity of protein load and transfer efficiency across the test samples.

Measurement of Tissue Nitrate Plus Nitrite

Kidney tissues (25%, wt/vol) were homogenized in 10 mM HEPES buffer, pH 7.4, containing 1 mM EDTA, 1 mM DTT, 10 μg/ml leupeptin, and 2 μg/ml aprotinin at 0–4°C with the aid of a Polytron homogenizer. Homogenates were centrifuged at 12,000 *g* for 5 min at 4°C, and the supernatant was used for the determination of total nitrate plus nitrite (NOx) using the purge system of a Sievers Instruments model 270B NO analyzer (Boulder, CO) as described previously (50).

Data Analysis

Regression analysis and a *t*-test were used in the statistical analysis of the data, which are presented as means ± SE. *P* values ≤0.05 were considered significant.

RESULTS

NOS Isotype Values

Aorta. iNOS and eNOS proteins were detectable in the aorta tissue preparations in both groups. The HU group exhibited a marked upregulation of iNOS protein expression in the aorta compared with the control group ($P < 0.01$). In contrast, aorta eNOS protein in the HU group was similar to that found in the control group (Fig. 1).

Heart. Both iNOS and eNOS proteins were expressed in the cardiac tissues of the study animals. Compared with the control group, the HU group showed a significant increase in heart tissue iNOS protein abundance ($P < 0.05$). However, no significant difference was found in cardiac tissue eNOS abundance between the HU and the control group (Fig. 2).

Kidney. Immunodetectable eNOS, iNOS, and nNOS proteins were present in the renal tissue preparations of all animals. As with the heart and aorta, kidney iNOS expression was significantly increased ($P < 0.01$) in the HU group. In addition, nNOS protein abundance was significantly elevated in the HU animals when compared with that found in the control group ($P < 0.01$). However, as with the other tissues, kidney eNOS protein expression was unaffected by HU treatment. Data are illustrated in Fig. 3.

Brain. Determination of nNOS protein revealed a significant elevation of nNOS protein abundance in the brain of HU animals compared with that in the control group ($P < 0.01$). Data are depicted in Fig. 4.

Tissue NOx Level

Kidney tissue NOx content in the HU group (1.08 ± 0.08 nmol/mg protein) was significantly higher than that found in the control group (0.77 ± 0.06 nmol/mg protein, $P < 0.05$). A significant correlation was found between kidney tissue NOx content and kidney tissue iNOS ($r = 0.809$, $P < 0.05$) and nNOS ($r = 0.902$, $P < 0.01$) but not eNOS [$r = 0.112$, P = not significant (NS)].

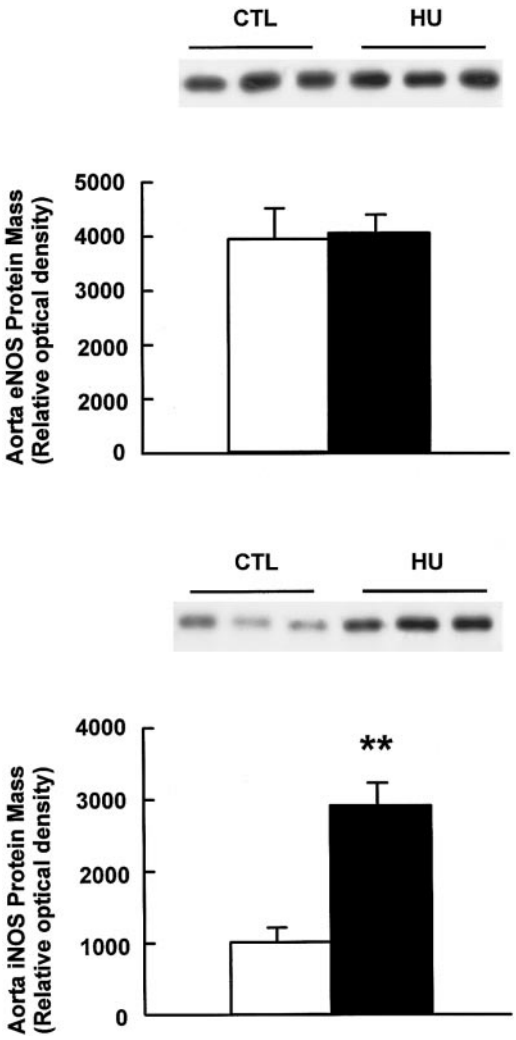

Fig. 1. Representative Western blots (3 animals from each group) and group data ($n = 6$ in each group) depicting aorta endothelial nitric oxide synthase (eNOS, *top*) and inducible NOS (iNOS, *bottom*) protein abundance in the hindlimb unloaded (HU) and control (CTL) groups. $^{**}P < 0.01$

Response to Norepinephrine and Aminoguanidine

Baseline systolic arterial blood pressure in the HU group (140 ± 20 mmHg) was not significantly different from that seen in the control group (147 ± 15 mmHg, P = NS). Likewise, baseline heart rates were comparable in the two groups (442 ± 19 beats/min and 439 ± 22 beats/min, respectively, P = NS). Norepinephrine administration resulted in an expected rise in arterial blood pressure in both groups. However, the HU group exhibited a significant attenuation of hypertensive response to norepinephrine administration compared with that seen in the control group ($P < 0.01$). Administration of the iNOS inhibitor aminoguanidine resulted in a significant rise in blood pressure in both groups. However, aminoguanidine administration elicited a significantly greater pressor response in the HU group compared with that in the control group (Fig. 5). These findings point to enhanced iNOS activity and

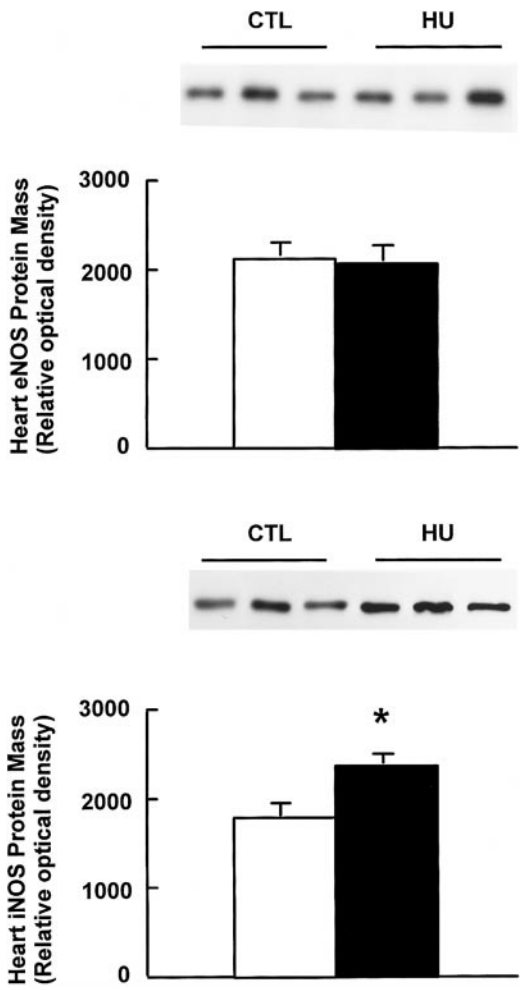

Fig. 2. Representative Western blots (3 animals from each group) and group data ($n = 6$ rats in each group) depicting heart eNOS (*top*) and iNOS (*bottom*) protein abundance in the HU and control groups. $^{*}P < 0.05$

provide functional evidence for the observed increase in iNOS abundance in the HU group.

DISCUSSION

The HU group exhibited a significant upregulation of iNOS protein expression in the aorta, heart, and the kidney. This was accompanied by increased nNOS protein expression in the brain and the kidney in the HU group. However, eNOS abundance was not altered by chronic HU in our animals. The elevation of renal iNOS and nNOS proteins was accompanied by a significant increase in the renal tissue content of stable NO metabolites (NOx). This observation points to increased NO production in the kidney. In fact, renal tissue NOx was significantly related to the corresponding iNOS and nNOS levels, which were elevated, but not with eNOS, which was unaffected by HU.

According to the traditional view, iNOS is not expressed under physiological conditions. Instead, iNOS is induced under certain pathological conditions, namely, during inflammatory states and, most dramatically, in septic shock. iNOS can also be induced experimentally both in vivo and in vitro by endotoxin and the proinflammatory cytokines, tumor necrosis factor-α, interleukin-1β, and interferon-γ (38). In addition to the classic induction pathway noted above, a low-level constitutive expression of iNOS has been recently demonstrated in several tissues, including kidney, heart, and blood vessel wall under normal conditions (1, 23, 24, 29). Moreover, dysregulation of constitutively expressed iNOS has been reported in several clinical and experimental conditions associated with disturbances of blood pressure, fluid, and electrolytes (27, 35, 47–49). For instance, we have shown downregulation of renal and vascular tissue iNOS in uremic

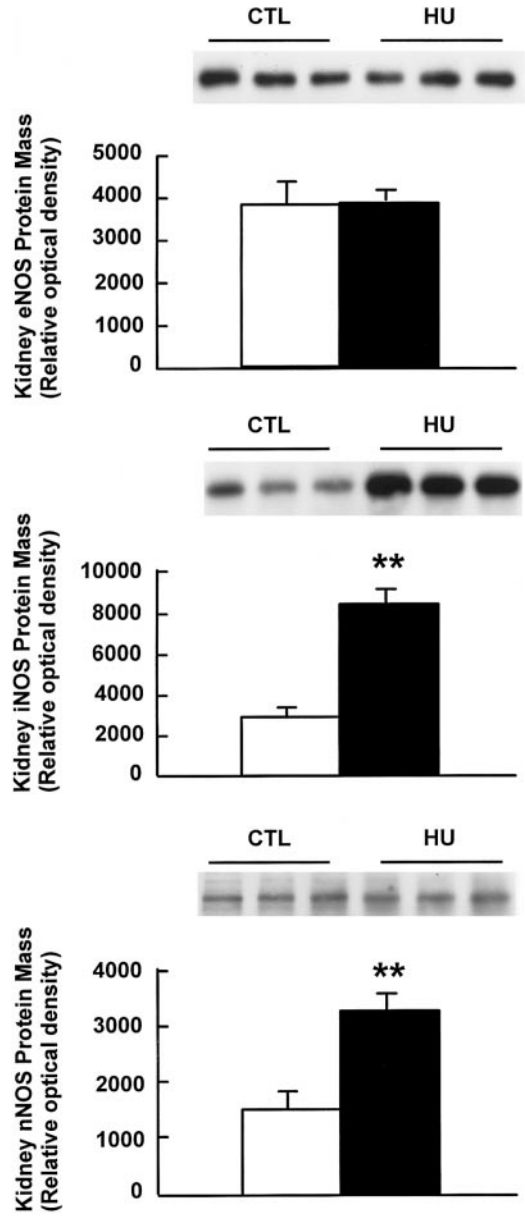

Fig. 3. Representative Western blots (3 animals from each group) and the corresponding group data ($n = 6$ rats in each group) illustrating eNOS (*top*), iNOS (*middle*), and neuronal NOS (nNOS; *bottom*) abundances in the kidney tissue preparations from the HU and the control groups. $^{**}P < 0.01$

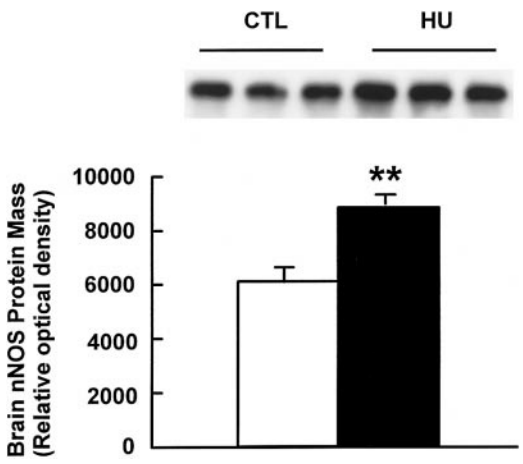

Fig. 4. Representative Western blot (3 animals from each group) and group data ($n = 6$ rats in each group) depicting brain nNOS protein abundance in the HU and control groups. $^{**}P < 0.01$

rats, cyclosporine-treated animals, and Dahl salt-sensitive rats (27, 48, 49), models which are characterized by hypertension and salt retention. In contrast, we have found marked upregulation of renal and vascular iNOS expression in young spontaneously hypertensive rats wherein hypertension is not associated with salt retention (47). Thus low-level constitutive expression of iNOS plays a physiologically regulatory role that is distinct from classic endotoxin/cytokine-mediated induction of iNOS leading to massive release of NO and other reactive species, hypotension, and cytotoxicity. We believe that upregulation of renal, vascular, and cardiac iNOS expression and of renal and brain nNOS expression in the HU group contributes to adaptation to simulated microgravity and the resulting cardiovascular deconditioning in this model. This viewpoint is supported by the observation that the HU animals exhibited a significantly greater hypertensive response to administration of the reputed iNOS inhibitor, aminoguanidine, than did the control group. The observed pressor response in the HU group points to increased iNOS-derived NO production in these animals, thus providing functional evidence for the observed increase in iNOS protein abundance. Together, these findings support the role of upregulation of iNOS expression in the adaptive vasodilatory response to simulated microgravity and hyporesponsiveness to vasoconstrictive agents previously shown by Purdy et al. (31, 34) and Delp et al. (8) and confirmed in the present study. Moreover, we have recently reported functional evidence suggesting that iNOS activity is elevated in the femoral artery of HU rats (34).

Aminoguanidine was used because it demonstrated selectivity for iNOS over eNOS and nNOS. For example, in our earlier study (34), 100 μM aminoguanidine had no effect on acetylcholine-mediated, endothelium-dependent relaxation of isolated arteries. Moreover, Wolff and Lubeskie (55) showed that aminoguanidine is 50–500 times more selective for iNOS over nNOS. In the present study, we measured NOS isotypes in the thoracic aorta, which is a conduit artery. Although small resistance arteries would have been more desirable, their use in this assay, which requires a substantial amount of tissue for protein extraction, was impractical. It is of interest that changes of eNOS and iNOS in the kidney and heart, tissues that are replete with resistance arteries and arterioles, paralleled those found in the aorta of HU animals. This observation suggests that findings in the aorta may mirror those of the small arteries and arterioles, which are the primary target of NO. Immunohistological studies are required to confirm this supposition.

In addition to upregulation of iNOS, the HU animals employed in the present study exhibited a marked upregulation of kidney nNOS protein expression. nNOS is normally expressed in different parts of the kidney, particularly in macula densa and endothelia of efferent arteriole (2, 25, 44, 54). nNOS-derived NO plays an important role in modulation of renal microvascular function and tubuloglomerular feedback (13–15, 20, 28, 40, 41, 46, 53). In addition, renal iNOS-derived NO is thought to play an important role in renal sodium handling. For instance, conditions marked by impaired renal sodium handling and salt sensitivity such as seen in Dahl salt-sensitive rats (27), cyclosporine-induced hypertension (49), and chronic renal failure (48) are accompanied by depressed iNOS expression in the kidney and other tissues. It is, therefore, conceivable that upregulations of renal iNOS and nNOS may facilitate renal sodium excretion and thus contribute to natriuresis and hypovolemia, which are known consequences of extended exposure to microgravity.

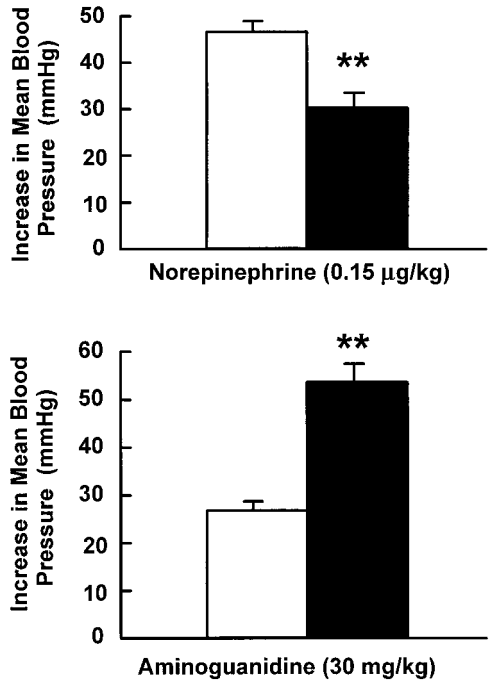

Fig. 5. Change in mean arterial pressure in response to intravenous bolus injections of norepinephrine [0.15 μg/kg (Sigma Chemical); *top*] and iNOS inhibitor aminoguanidine [30 mg/kg (Sigma Chemical); *bottom*] in the HU and control groups. $^{**}P < 0.01$; $n = 6$ rats in each group.

The HU animals employed in the present study exhibited a marked upregulation of brain nNOS abundance. nNOS is normally expressed in various regions of the brain and is thought to be involved in neurogenic control of blood pressure by inhibiting central sympathetic outflow (4, 11, 43, 51). Consequently, nNOS-derived NO in the brain is considered to exert a blood pressure-lowering influence. If true, upregulation of brain nNOS may potentially contribute to orthostatic intolerance by suppressing the central sympathetic outflow following extended exposure to microgravity. In support of this notion, Moffitt et al. (22) have recently shown a marked attenuation of basal lumbar and renal sympathetic nerve activities in the HU animals compared with the control rats. They have further demonstrated a significant attenuation of lumbar and renal sympathetic system activity in response to a hypotensive challenge in these animals (22). It is of interest that brain nNOS expression is increased in various models of hypertension, including rats with chronic renal failure (56), salt-loaded, salt-sensitive Dahl rats (27), and rats with aortic coarctation-induced hypertension (3). These observations suggest the possible role of elevated pressure in regulation of brain nNOS expression. Although the HU animals did not have systemic hypertension, the shift in gravitational forces are known to markedly raise blood pressure in the cranial circulation (12). This local rise in the cranial blood pressure may, therefore, be responsible for the observed upregulation of brain nNOS protein expression in HU animals in a manner analogous to that seen in animals with systemic hypertension.

Although Western blot analysis demonstrated upregulations of iNOS and nNOS in the given tissues, it did not identify specific regions or cell types contributing to this phenomenon. Immunohistological studies are required to address this issue.

Numerous authors (7–9, 31, 34, 42) have used the HU model of the present study to simulate microgravity and have compared HU rats to unsuspended, separately caged controls. It must be acknowledged that the stress associated with the tail harness itself could have been responsible for the cardiovascular effects of this model. However, this seems unlikely on the following grounds. Murison et al. (26) argued that the effects of HU are not a result of an increase in stress. He found only an initial transient increase in plasma corticosterone. In addition, the absence of cardiac hypertrophy suggests that the cardiovascular system was not stressed by HU (52). Kahwaji et al. (16) also found that HU substantially reduced the maximal vascular contraction to norepinephrine but had no effect on that to serotonin. This also argues against a nonspecific effect of HU, such as stress. Although an effect of HU, independent of its hemodynamic effects, is unlikely, this possibility cannot be excluded with certainty. Future experiments comparing harnessed rats in the hindlimb elevated vs. horizontal positions will be required to resolve this issue.

In conclusion, extended exposure to simulated microgravity in the HU animals resulted in marked upregulations of renal, vascular, and cardiac iNOS protein expression and enhanced renal and brain nNOS protein expression and tissue NOx content. Upregulation of iNOS and nNOS expressions in the HU animals was coupled with a depressed pressor response to norepinephrine and an enhanced pressor response to the iNOS inhibitor aminoguanidine. The latter findings provide functional evidence for enhanced iNOS-derived NO activity in the HU animals. Upregulation of iNOS and nNOS may play an important role in chronic adaptation to microgravity and subsequent orthostatic intolerance on reexposure to normal gravity. If true, short-term iNOS inhibition may ameliorate the orthostatic intolerance associated with extended exposure to microgravity. Further studies are needed to explore the usefulness of this therapeutic strategy.

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REFERENCES

1. **Ahn KY, Mohaupt MG, Madsen KM, and Kone BC.** In situ hybridization localization of mRNA encoding inducible nitric oxide synthase in rat kidney. *Am J Physiol Renal Fluid Electrolyte Physiol* 267: F748–F757, 1994.
2. **Bachmann S, Bosse HM, and Mundel P.** Topography of nitric oxide synthesis by localizing constitutive NO synthases in mammalian kidney. *Am J Physiol Renal Fluid Electrolyte Physiol* 268: F885–F898, 1995.
3. **Barton CH, Biggs TE, Baker ST, Bowen H, and Atkinson PG.** Nramp1: a link between intracellular iron transport and innate resistance to intracellular pathogens. *J Leukoc Biol* 66: 757–762, 1999.
4. **Bredt DS, Hwang PM, and Snyder SH.** Localization of nitric oxide synthase indicating a neural role for nitric oxide. *Nature* 347: 768–770, 1990.
5. **Buckey JC Jr, Lane LD, Levine BD, Watenpaugh DE, Wright SJ, Moore WE, Gaffney FA, and Blomqvist CG.** Orthostatic intolerance after spaceflight. *J Appl Physiol* 81: 7–18, 1996.
6. **Charles JB and Lathers CM.** Cardiovascular adaptation to spaceflight. *J Clin Pharmacol* 31: 1010–1023, 1991.
7. **Delp MD, Brown M, Laughlin MH, and Hasser EM.** Rat aortic vasoreactivity is altered by old age and hindlimb unloading. *J Appl Physiol* 78: 2079–2086, 1995.
8. **Delp MD, Holder-Binkley T, Laughlin MH, and Hasser EM.** Vasoconstrictor properties of rat aorta are diminished by hindlimb unweighting. *J Appl Physiol* 75: 2620–2628, 1993.
9. **Geary GG, Krause DN, Purdy RE, and Duckles SP.** Simulated microgravity increases myogenic tone in rat cerebral arteries. *J Appl Physiol* 85: 1615–1621, 1998.
10. **Glen JB and Scott WN.** Carbon dioxide euthanasia of cats. *Br Vet J* 129: 471–479, 1973.
11. **Harada S, Tokunaga S, Momohara M, Masaki H, Tagawa T, Imaizumi T, and Takeshita A.** Inhibition of nitric oxide formation in the nucleus tractus solitarius increases renal sympathetic nerve activity in rabbits. *Circ Res* 72: 511–516, 1993.
12. **Hargens AR, Watenpaugh DE, and Breit GA.** Control of circulatory function in altered gravitational fields. *Physiologist* 35, *Suppl* 1: S80–S83, 1992.
13. **Ichihara A, Inscho EW, Imig JD, and Navar LG.** Neuronal nitric oxide synthase modulates rat renal microvascular function. *Am J Physiol Renal Physiol* 274: F516–F524, 1998.
14. **Ichihara A and Navar LG.** Neuronal NOS contributes to biphasic autoregulatory response during enhanced TGF activity. *Am J Physiol Renal Physiol* 277: F113–F120, 1999.
15. **Ito S and Ren Y.** Evidence for the role of nitric oxide in macula densa control of glomerular hemodynamics. *J Clin Invest* 92: 1093–1098, 1993.

16. **Kahwaji CI, Sangha DS, Sheibani S, Ashori M, Nguyen H, Kim Y, and Purdy RE.** Simulated microgravity-induced vascular hyporesponsiveness: role of signal transduction. *Proc West Pharmacol Soc* 42: 9–12, 1999.
17. **Kanno K, Hirata Y, Emori T, Ohta K, Eguchi S, Imai T, and Marumo F.** L-Arginine infusion induces hypotension and diuresis/natriuresis with concomitant increased urinary excretion of nitrite/nitrate and cyclic GMP in humans. *Clin Exp Pharmacol Physiol* 19: 619–625, 1992.
18. **Lahera V, Salom MG, Fiksen-Olsen MJ, and Romero JC.** Mediatory role of endothelium-derived nitric oxide in renal vasodilatory and excretory effects of bradykinin. *Am J Hypertens* 4: 260–262, 1991.
19. **Majid DS, Williams A, and Navar LG.** Inhibition of nitric oxide synthesis attenuates pressure-induced natriuretic responses in anesthetized dogs. *Am J Physiol Renal Fluid Electrolyte Physiol* 264: F79–F87, 1993.
20. **Mattson DL and Bellehumeur TG.** Neural nitric oxide synthase in the renal medulla and blood pressure regulation. *Hypertension* 28: 297–303, 1996.
21. **Mattson DL, Lu S, Nakanishi K, Papanek PE, and Cowley AW Jr.** Effect of chronic renal medullary nitric oxide inhibition on blood pressure. *Am J Physiol Heart Circ Physiol* 266: H1918–H1926, 1994.
22. **Moffitt JA, Foley CM, Schadt JC, Laughlin MH, and Hasser EM.** Attenuated baroreflex control of sympathetic nerve activity after cardiovascular deconditioning in rats. *Am J Physiol Regulatory Integrative Comp Physiol* 274: R1397–R1405, 1998.
23. **Mohaupt MG, Elzie JL, Ahn KY, Clapp WL, Wilcox CS, and Kone BC.** Differential expression and induction of mRNAs encoding two inducible nitric oxide synthases in rat kidney. *Kidney Int* 46: 653–665, 1994.
24. **Morrissey JJ, McCracken R, Kaneto H, Vehaskari M, Montani D, and Klahr S.** Location of an inducible nitric oxide synthase mRNA in the normal kidney. *Kidney Int* 45: 998–1005, 1994.
25. **Mundel P, Bachmann S, Bader M, Fischer A, Kummer W, Mayer B, and Kriz W.** Expression of nitric oxide synthase in kidney macula densa cells. *Kidney Int* 42: 1017–1019, 1992.
26. **Murison R, Overmier JB, and Skoglund EJ.** Serial stressors: prior exposure to a stressor modulates its later effectiveness on gastric ulceration and corticosterone release. *Behav Neural Biol* 45: 185–195, 1986.
27. **Ni Z, Oveisi F, and Vaziri ND.** Nitric oxide synthase isotype expression in salt-sensitive and salt-resistant Dahl rats. *Hypertension* 34: 552–557, 1999.
28. **Ollerstam A, Pittner J, Persson AE, and Thorup C.** Increased blood pressure in rats after long-term inhibition of the neuronal isoform of nitric oxide synthase. *J Clin Invest* 99: 2212–2218, 1997.
29. **Park CS, Park R, and Krishna G.** Constitutive expression and structural diversity of inducible isoform of nitric oxide synthase in human tissues. *Life Sci* 59: 219–225, 1996.
30. **Plato CF, Stoos BA, Wang D, and Garvin JL.** Endogenous nitric oxide inhibits chloride transport in the thick ascending limb. *Am J Physiol Renal Physiol* 276: F159–F163, 1999.
31. **Purdy RE, Duckles SP, Krause DN, Rubera KM, and Sara D.** Effect of simulated microgravity on vascular contractility. *J Appl Physiol* 85: 1307–1315, 1998.
32. **Radermacher J, Klanke B, Schurek HJ, Stolte HF, and Frolich JC.** Importance of NO/EDRF for glomerular and tubular function: studies in the isolated perfused rat kidney. *Kidney Int* 41: 1549–1559, 1992.
33. **Rozcniak A and Burns KD.** Nitric oxide stimulates guanylate cyclase and regulates sodium transport in rabbit proximal tubule. *Am J Physiol Renal Fluid Electrolyte Physiol* 270: F106–F115, 1996.
34. **Sangha DS, Vaziri ND, Ding Y, and Purdy RE.** Vascular hyporesponsiveness in simulated microgravity: role of nitric oxide-dependent mechanisms. *J Appl Physiol* 88: 507–517, 2000.
35. **Seftel AD, Vaziri ND, Ni Z, Razmjouei K, Fogarty J, Hampel N, Polak J, Wang RZ, Ferguson K, Block C, and Haas C.** Advanced glycation end products in human penis: elevation in diabetic tissue, site of deposition, and possible effect through iNOS or eNOS. *Urology* 50: 1016–1026, 1997.
36. **Stoos BA, Carretero OA, Farhy RD, Scicli G, and Garvin JL.** Endothelium-derived relaxing factor inhibits transport and increases cGMP content in cultured mouse cortical collecting duct cells. *J Clin Invest* 89: 761–765, 1992.
37. **Stoos BA, Garcia NH, and Garvin JL.** Nitric oxide inhibits sodium reabsorption in the isolated perfused cortical collecting duct. *J Am Soc Nephrol* 6: 89–94, 1995.
38. **Thiemermann C.** Nitric oxide and septic shock. *Gen Pharmacol* 29: 159–166, 1997.
39. **Thomason DB, Herrick RE, Surdyka D, and Baldwin KM.** Time course of soleus muscle myosin expression during hindlimb suspension and recovery. *J Appl Physiol* 63: 130–137, 1987.
40. **Thorup C, Erik A, and Persson G.** Macula densa derived nitric oxide in regulation of glomerular capillary pressure. *Kidney Int* 49: 430–436, 1996.
41. **Thorup C and Persson AE.** Inhibition of locally produced nitric oxide resets tubuloglomerular feedback mechanism. *Am J Physiol Renal Fluid Electrolyte Physiol* 267: F606–F611, 1994.
42. **Tipton CM and Sebastian LA.** Dobutamine as a countermeasure for reduced exercise performance of rats exposed to simulated microgravity. *J Appl Physiol* 82: 1607–1615, 1997.
43. **Toda N, Kitamura Y, and Okamura T.** Neural mechanism of hypertension by nitric oxide synthase inhibitor in dogs. *Hypertension* 21: 3–8, 1993.
44. **Tojo A, Gross SS, Zhang L, Tisher CC, Schmidt HH, Wilcox CS, and Madsen KM.** Immunocytochemical localization of distinct isoforms of nitric oxide synthase in the juxtaglomerular apparatus of normal rat kidney. *J Am Soc Nephrol* 4: 1438–1447, 1994.
45. **Tojo A, Guzman NJ, Garg LC, Tisher CC, and Madsen KM.** Nitric oxide inhibits bafilomycin-sensitive H^+-ATPase activity in rat cortical collecting duct. *Am J Physiol Renal Fluid Electrolyte Physiol* 267: F509–F515, 1994.
46. **Vallon V and Thomson S.** Inhibition of local nitric oxide synthase increases homeostatic efficiency of tubuloglomerular feedback. *Am J Physiol Renal Fluid Electrolyte Physiol* 269: F892–F899, 1995.
47. **Vaziri ND, Ni Z, and Oveisi F.** Upregulation of renal and vascular nitric oxide synthase in young spontaneously hypertensive rats. *Hypertension* 31: 1248–54, 1998.
48. **Vaziri ND, Ni Z, Wang XQ, Oveisi F, and Zhou XJ.** Downregulation of nitric oxide synthase in chronic renal insufficiency: role of excess PTH. *Am J Physiol Renal Physiol* 274: F642–F649, 1998.
49. **Vaziri ND, Ni Z, Zhang YP, Ruzics EP, Maleki P, and Ding Y.** Depressed renal and vascular nitric oxide synthase expression in cyclosporine-induced hypertension. *Kidney Int* 54: 482–491, 1998.
50. **Vaziri ND, Oveisi F, and Ding Y.** Role of increased oxygen free radical activity in the pathogenesis of uremic hypertension. *Kidney Int* 53: 1748–1754, 1998.
51. **Vincent SR and Kimura H.** Histochemical mapping of nitric oxide synthase in the rat brain. *Neuroscience* 46: 755–784, 1992.
52. **Wang C, Chao C, Chen LM, Chao L, and Chao J.** High-salt diet upregulates kininogen and downregulates tissue kallikrein expression in Dahl-SS and SHR rats. *Am J Physiol Renal Fluid Electrolyte Physiol* 271: F824–F830, 1996.
53. **Wilcox CS and Welch WJ.** TGF and nitric oxide: effects of salt intake and salt-sensitive hypertension. *Kidney Int Suppl* 55: S9–S13, 1996.
54. **Wilcox CS, Welch WJ, Murad F, Gross SS, Taylor G, Levi R, and Schmidt HH.** Nitric oxide synthase in macula densa regulates glomerular capillary pressure. *Proc Natl Acad Sci USA* 89: 11993–11997, 1992.
55. **Wolff DJ and Lubeskie A.** Aminoguanidine is an isoform-selective, mechanism-based inactivator of nitric oxide synthase. *Arch Biochem Biophys* 316: 290–301, 1995.
56. **Ye S, Nosrati S, and Campese VM.** Nitric oxide (NO) modulates the neurogenic control of blood pressure in rats with chronic renal failure (CRF). *J Clin Invest* 99: 540–548, 1997.